Developing a delivery system for the control of plant diseases: from leaf pathogens control to grapevine trunk diseases control in the nursery

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Grapevine trunk diseases (GTDs) are a major threat for viticulture, in all grape-growing countries. They are all caused by fungal pathogens infecting the vines by wounds. The fungal agents cause wood decay, cankers or infections of the vascular tissue causing different symptoms. The main diseases identified in Europe are the ones within the Esca Complex of diseases: vascular pathogens (Pch, Pmin) and wood rot agents (Fmed) surely interacting with the main canker agents (Bot species).
The management of GTDs has several critical aspects, and one of the main ones is the lack of suitable treatment with low toxicity but good efficacy. This suggested to investigate the applicability in plant protection of micro-structured inorganic crystals based on Carbonate HydroxyApatite (CHA), already in use in the medical field as innovative delivery system, to enhance the biological activity and the efficacy *in planta* of traditional substances, such as the copper(II) compounds.

AIMS

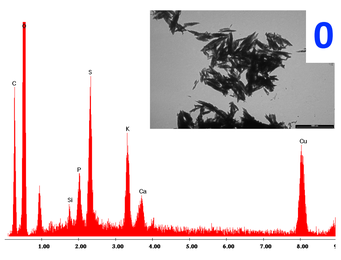
The research is aimed to:

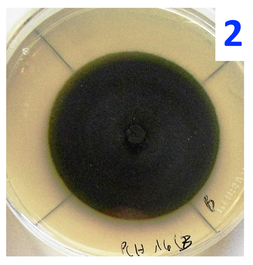
- apply the CHA to two copper(II) compounds, verifying the improvement of the copper efficiency in a *Vitis vinifera* protective greenhouse test against *Plasmopara viticola*;
- investigate the biological activity of both copper(II) compounds, pure and formulated with CHA, on the growth *in vitro* of two GTDs related pathogens;
- evaluate the fungicide activity *in vivo* of the same formulations and the potential application in the control of GTDs related pathogens in grapevine propagating material;
- evaluate the potential stimulation of the defence responses related to GTDs in *V. vinifera*, through foliar applications of the same formulations;
- understand the role of copper(II) based treatments and its optimisations on controlling GTDs.

MATERIALS AND METHODS

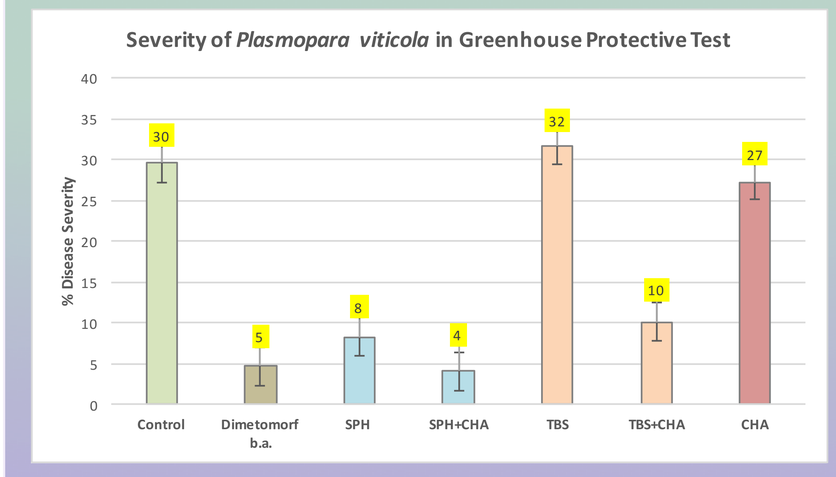
Several formulations were prepared functionalizing CHA with two different copper(II) compounds (fig. 0): tribasic sulphate (TBS) and sulphate pentahydrate (SPH). The formulations were applied in the following tests:

1) **Greenhouse protective test** on potted vines (*V. vinifera* L.) inoculated with *Plasmopara viticola,* in order to detect the disease severity after 7 days, comparing the protection achieved by the formulations and vs a fungicide and the untreated control;
2) ***In vitro* test** to evaluate the growth inhibition of GTD related pathogens, such as *Phaeomoniella chlamydospora* (Pch) and *Neofusicoccum parvum* (Np);
3) **Nursery protective test** on grapevine propagating material (rootstocks cv. Kober 5BB and scions cv. Chardonnay) hydrated with water solutions containing the CHA-copper(II) based formulations and inoculated with spores of Pch before to be grafted, callused, planted in the field and finally harvested for the pathogen reisolation;
4) ***In planta* gene regulation test** on grapevine scions (cv. Chardonnay), grown in greenhouse, inoculated with Np, and treated on the leaves with the same formulations before collecting foliar samples at 8hpt for RNA extractions and RT/qPCR analysis.





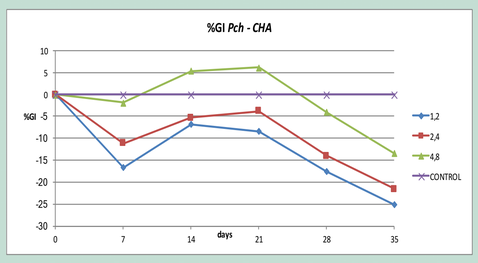
GREENHOUSE PROTECTIVE TEST AGAINST *Plasmopara viticola*

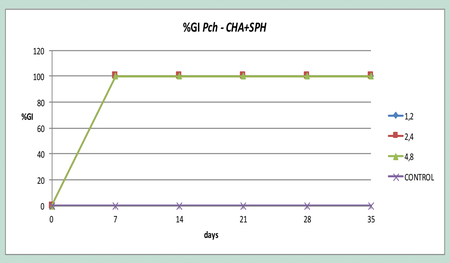


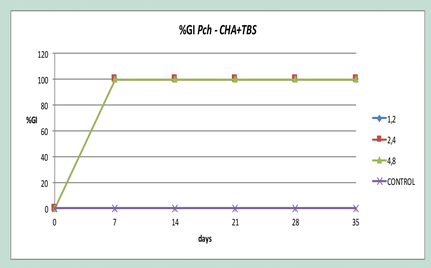
FOLIAR APPLICATIONS based on 0.025% Cu (SPH and TBS)

The greenhouse protective test showed a positive contribution of CHA in enhancing the activity of copper against the pathogen: for TBS there is a high difference also due to the low activity of the technical TBS, while for SPH, the CHA reduced the phytotoxic effect of the technical SPH. CHA was not significantly different from the control.

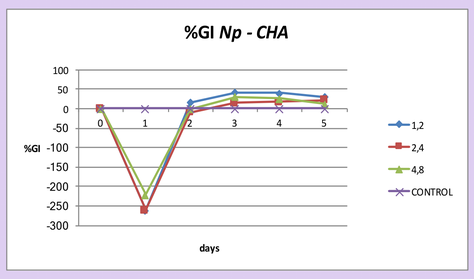
IN VITRO TEST ON *Pch* AND *Np*

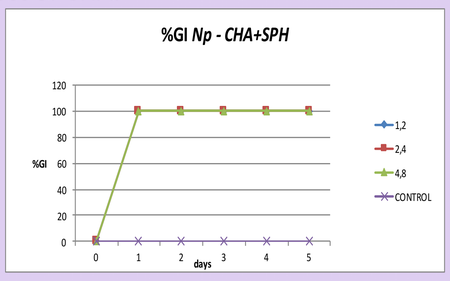


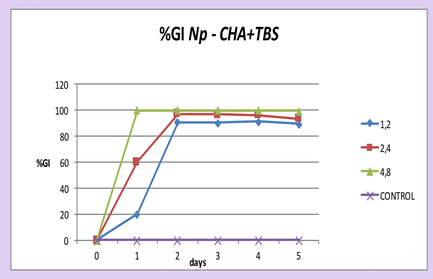




Results are expressed in % of Growth Inhibition (GI) at 3 concentrations of testing formulation: 1.2 – 2.4 – 4.8%.
CHA showed a stimulant effect on Pch (as reported in the greenhouse test), while the copper based formulations inhibited completely the Pch growth in the first week, obviously as a consequence of the presence of copper(II) ions, which seemed to mask the stimulant effect of CHA.







CHA showed the inhibition effect on the growth of Np only at the very initial stage of the growth, while already in the second day it seemed to stimulate its growth. The formulations based on copper(II) showed the same results reported for Pch with a lower inhibition of Np achieved by CHA+TBS. Globally it was observed the stimulant and non fungitoxic effect of CHA and the high and stable growth inhibition for both CHA+SPH and CHA+TBS.

IN PLANTA GENE REGULATION TEST (1)

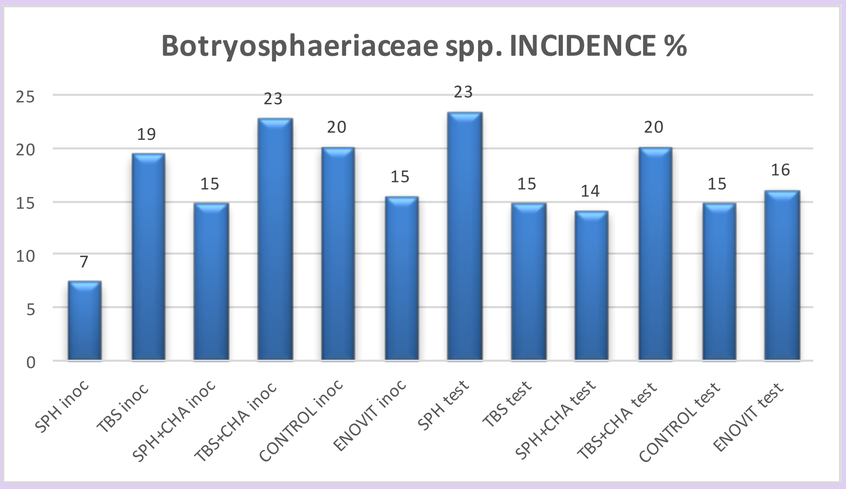
Expression 8hpt	CTRL	CHA	TBS	CHA + TBS	SPH	CHA + SPH
CHIT4C	1.08	3.53	10.81	2.91	65.93	37.46
GLU	1.02	3.95	14.69	4.08	58.66	68.08
IFRL4	1.01	5.38	0.48	3.76	0.84	0.41
LHCA3	1.02	0.87	0.44	0.73	0.14	0.08
LOX	1.03	0.46	0.67	0.77	2.14	0.97
PAL	1.02	0.63	5.01	1.06	24.27	17.30
PGIP	1.02	1.29	1.58	1.15	2.29	1.28
POX	1.00	0.78	0.96	0.94	0.79	0.57
PPO	1.00	1.05	1.09	1.59	1.67	2.39
PR1	0.96	11.85	23.98	2.75	126.20	217.52
PR10	1.05	2.40	23.69	5.67	186.13	238.42
PR6	1.25	1.03	1.92	1.33	21.87	4.62
PSBP1	1.03	1.21	0.82	0.96	0.27	0.18
STS	1.05	0.67	11.65	2.61	64.11	75.32

high defence responses induction/repression

(same formulations and amounts applied in nursery during wood hydration)

NURSERY PROTECTIVE TEST TO CONTROL GTDs PATHOGENS

INCIDENCE % of Botryosphaeriaceous species naturally present in the propagating material: only the SPH showed a fungi-toxic effect compared to the control, but only in the inoculated material.

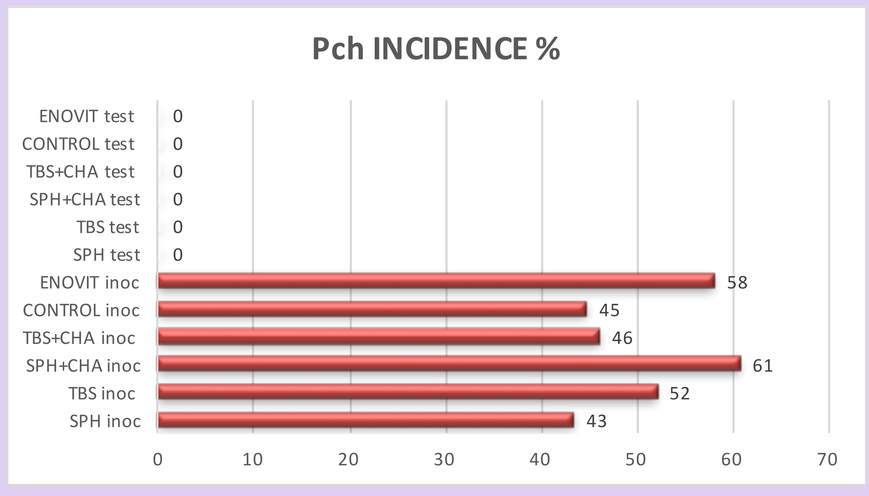


WRONG TARGET ???

IN PLANTA GENE REGULATION TEST (2)

Expression 8hpt	CTRL Np	CHA Np	TBS Np	CHA+TBS Np	SPH Np	CHA+SPH Np
CHIT4C	1.08	1.61	5.83	13.90	72.07	30.86
GLU	0.74	0.94	8.89	14.60	35.22	51.08
IFRL4	12.11	1.44	1.28	12.64	1.63	1.67
LHCA3	1.00	0.95	0.27	0.59	0.08	0.15
LOX	0.88	0.88	1.26	8.57	1.65	3.66
PAL	0.89	0.70	4.82	15.92	11.61	13.69
PGIP	1.08	0.77	1.07	18.58	30.39	2.50
POX	0.98	0.43	0.91	1.11	1.26	0.70
PPO	1.52	1.24	1.63	2.01	7.86	2.88
PR1	4.40	1.55	44.56	48.84	169.81	206.49
PR10	1.73	0.90	7.51	9.17	187.57	121.25
PR6	1.88	1.23	2.46	19.97	30.88	10.32
PSBP1	1.01	0.93	0.48	0.78	0.14	0.27
STS	0.88	1.39	22.51	16.77	43.90	49.75

high defence responses induction/repression on plants inoculated with Np



INCIDENCE % of Pch: no colonies were reisolated from the test material, while a high incidence of Pch was detected in all the conditions with no significant effect in comparison to the control.

CONCLUSIONS

Thorough an approach based on consecutive trials, the potential biological activity of two copper compounds was evaluated, showing very promising results against *Plasmopara viticola*. The same formulations showed a significant fungitoxic activity *in vitro*, against GTDs pathogens, but the effective concentrations did not show the same efficiency *in planta* on protecting the grapevine propagation material.

The potential activity on GTDs control was revealed by the same formulations as significant induction/repression of the defense responses related genes, in both tested conditions (non inoculated/inoculated Np), suggesting further investigations for potential field applications.